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MILK FAT HYDROLYSIS IN CHEESE

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

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ABSTRACT

The hydrolysis of milk fat in Cheddar cheese which occurs during ripening results in the liberation of free fatty acids. The lower molecular weight fatty acids (C4 to C8) have an extremely low flavor threshold which suggests their important contribution to the characteristic Cheddar cheese flavor. The water solubility and high volatility of the lower molecular weight acids, up to and including caproic, present difficulties of analysis. Solvent extraction and column chromatographic techniques which have been employed do not prevent volatile acid losses. A two-step gas-chromatographic analytical procedure was developed, which apparently overcomes this difficulty. A total of 43 mild, medium and old commercial Canadian Cheddar cheeses were analyzed and shown to contain a fairly constant butterfat content of approximately 35.0%. Fat acidity was shown to increase with increased aging and ripening of the cheese, old cheeses exhibiting a mean fat acid content one-third higher than mild cheeses. The water soluble, steam volatile acids in Cheddar cheese showed a wide range of values in the mild, medium and old cheeses examined. The mean volatile acidity was highest in the old cheeses and lowest in the mild ones. The mean butyric and caproic acid levels in the old cheeses were two and a half times greater than in the mild samples while only a slight increase in acetic acid took place from mild to old. The ratio of butyric to acetic in the old cheese was 1:3 compared to 1:6 in the mild cheese. Unclean cheese showed mean fat and volatile acidity levels similar to those found in mild cheese. The ratio of butyric acid to acetic

was 1:6. Rancid cheese showed a very high butyric acid content of about the same level as in the old cheese but a very low acetic acid level. The ratio of butyric acid to acetic acid in rancid cheese was approximately 1.0:1.5. The high levels and ratios of lower fatty acids compared to the acetic acid level found in old and rancid cheeses suggests the significance of these acids in Cheddar cheese flavor. A certain amount of hydrolysis would appear to be necessary to impart the desirable characteristic flavor but if hydrolysis proceeds too far a rancid condition develops. Both the fat acidity and volatile acidity developed slowly in Cheddar cheese made with pasteurized milk in comparison with that made with unpasteurized milk.

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To my wife for her encouragement,

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INTRODUCTION

Canadian Cheddar cheese enjoys an enviable reputation. While the flavor of Cheddar cheese is variable, our senses of taste and smell are able to detect characteristics which distinguish Cheddar from other types of cheese. No chemical or physical measurement has been developed which will detect or measure the characteristic Cheddar flavor, nor has it been discovered how the characteristic flavor is formed.

In many countries considerable research has been done on the changes that take place in Cheddar as it matures and much has been learned about the agents responsible for these changes. It is safe to say, however, that the mystery of typical Cheddar cheese flavor remains unsolved.

The development of chromatographic techniques in 1944 spurred flavor research on cheese in most of the principal dairy countries of the world. Most research workers are in agreement that amino acids and fatty acids contribute to Cheddar cheese flavor.

A review of the literature points out the lack of published information on the ratios and amounts of free acids in Cheddar cheese. In view of the very low flavor threshold of the lower volatile free acids, and, in particular the fatty acid butyric, it would seem that such a study would be of considerable interest.

There is little doubt that the volatile acids, from acetic to capric, contribute to the flavor of Cheddar cheese. While acetic and propionic are products of bacterial fermentation, butyric, caproic,

caprylic and capric are mostly the result of lipolysis. The isolation and quantitative analysis of these acids has presented problems. One of the difficulties results from the fact that the acids up to and including caproic are water soluble, whereas caprylic and capric are not. Another difficulty arises from the fact that these acids are extremely volatile.

A method of analysis using gas-liquid chromatography has been developed which quantitatively measures the free volatile acids obtained from Cheddar cheese. This thesis reports the results of analyses of a number of cheeses, using a direct-distillation, gas-chromatographic technique not reported previously.

REVIEW OF LITERATURE

Fat Hydrolysis and Cheese Flavor.

When cheese ripens the fat of the cheese may become hydrolyzed. Stadhouders and Mulder (47) report that this was observed over sixty years ago by Van Slyke (44), Windish (50) and Orla Jensen (37). Fat hydrolysis is a normal process in cheese. Some of the fatty acids liberated have a penetrating smell. It is reasonable to suspect that fatty acids, such as butyric, caproic, caprylic and capric contribute to the flavor of cheese. One can often smell the fatty acids when tasting ripening cheese.

While there has been considerable material written on free fatty acids in cheese, the literature on fat hydrolysis and the relation of free fatty acids to the flavor of cheese is very confusing. Lane and Hammer (31) showed that, for Cheddar cheese, pasteurization of the milk caused an appreciable decrease in the amount of fat hydrolyzed in the cheese. They further showed that cheese made from raw homogenized cream mixed with skimmilk (raw or pasteurized) commonly developed a rancid flavor early during ripening. The condition tended to disappear as ripening progressed, and the cheese was more satisfactory in flavor than that made from pasteurized milk. They attributed the fat hydrolysis in Cheddar cheese largely to the action of milk lipase, an enzyme destroyed at pasteurization temperatures. They reported that the addition

of rennet paste and of desiccated mammary tissues to the milk caused an increase in the amount of fat hydrolysis in cheese.

Hlynka, Hood and Gibson (18) have reported that the vigorous agitation of raw milk caused a typical rancid flavor in the cheese.

Peterson, et al. (42) claimed that true milk lipase is not active in cheese. They stated that the lipase activity comes from the rennet and from bacteria but not from the milk, since the lipase then known, with pH optimum at 8 - 9, was inactivated at the pH of the cheese. Albrecht and Jaynes (1) also discussed the lipolytic activity of Cheddar cheese and suggested that an acid lipase of milk studied by them may be active in the cheese. The possibility of an acid lipase would seem worthy of further consideration.

Using the method of Peterson (41), no milk lipase activity in cheese was detected by Lubert, Smith and Thornton (33).

Alford and Frazier (2) reported a development of cheese flavor after the addition of certain strains of micrococci which contained lipase and grew well in young cheese. They did not give any figures on the hydrolysis of fat, confining their study to checking flavor of the cheese.

Hood, et al. (20) isolated fat-splitting bacteria from corn silage and from milk of bad quality. When added to cheese milk, these bacteria gave rise to rancid cheese. They did not indicate the type of bacteria responsible.

Lane and Hammer (31) and Harper and Gould (13) made reference to rennet preparations having an influence on the lipolysis of cheese fat. Stadhouders and Mulder (47) reported that rennet used in the Netherlands had no lipolytic activity. They also showed that fat hydrolysis occurred in cheese made from milk with almost no lipolytic microorganisms. They attributed the hydrolysis which occurred in the cheese from the aseptically-drawn milk to lipases which were secreted by the udder cells. Cheese made from aseptically-drawn milk, but subsequently contaminated with a normal flora from pails and containers that had not been cleaned over-carefully, showed more fat hydrolysis and a more pronounced cheese flavor. Their work showed that in cheese a few weeks old almost no living fat-hydrolyzing microorganisms were present. The lipolytic enzymes, however, remained active and they assumed these lipases worked independently of the living cell. Their view was later supported when sterile extracts from lipolytic bacteria of milk, added to pasteurized milk, caused fat hydrolysis in cheese manufactured from it. They made the further observation that bacteria which are important in the cheese ripening process do not necessarily grow in cheese and that it is important to pay attention to the bacteria of the milk and the very young cheese.

In a later report, Stadhouders and Mulder (48) studied the microorganisms involved in the hydrolysis of fat in the interior of cheese. The isolated strains of Serrateae, Pseudomonas and Achromobacteriaceae were found to be of importance in connection with fat

hydrolysis in cheese. These bacteria do not grow in cheese but die out very soon, leaving behind a lipolytic enzyme. Cultures of these bacteria, often occurring in large numbers on milking equipment, caused an important increase in the acidity of fat when added to cheese milk. The fatty acids thus liberated contributed significantly to the flavor of the cheese and were reported to cause a very strong off-flavor when they were produced in too large quantities. It was stated that the number of these bacteria present in normal raw milk was at times sufficient to have an influence on fat hydrolysis and on the flavor of the cheese.

Mabbitt (34) pointed out that since milk contains all the chemical substances from which cheese flavor is ultimately derived, it is surprising that there is not more information on the effect of changes in milk composition on the formation of Cheddar flavor. It has been observed that no typical flavor develops in Cheddar cheese made from skimmilk (35). This suggests that milk fat is the substrate for typical cheese flavor. It has also been found that increasing the lipolysis in the cheese, by the addition of enzymes or homogenization, can improve the flavor (7) but excessive lipolysis causes bitterness or rancidity.

If lipolysis is important in cheese flavor formation, then so probably is the composition of the milk fat. Since this can be varied by altering the feed, plane of nutrition and environment, and since it is also dependent on the stage of lactation of the cow (23), investigation of the effect of fat composition on Cheddar flavor is desirable.

Kristoffersen and Gould (27) made an extensive investigation of possible flavor compounds of a number of commercial Cheddar cheeses. They reported the amount of hydrogen sulphide was high in good cheeses while the ammonia and free fatty acid content was low. Cheeses made from raw milk seemed to contain more hydrogen sulphide and free amino acids, while the content of free fatty acids, oddly enough, was lower than in cheese made from pasteurized milk.

In a later study by Kristoffersen and Gould (28), the influence of hydrogen sulphide and free fatty acids was further investigated. They reported that Cheddar made from raw milk developed characteristic flavor earlier than cheese from good quality pasteurized milk, but after six months both kinds of cheese had the same flavor. Cheese made from inferior quality pasteurized milk had a lower score. The intensity of the Cheddar flavor was reported to be dependent on the molar ratio of C5 and higher acids together with acetic acid and hydrogen sulphide.

Kristoffersen and Nelson (29) investigated the production of hydrogen sulphide by Lactobacillus casei and its possible role in the formation of Cheddar cheese flavor. Those strains which showed the greatest activity were isolated from the best cheese. Cheeses with the highest relative concentration of hydrogen sulphide had the highest score for typical Cheddar flavor.

Volatile aldehydes and ketones have been found in Cheddar cheese. The work of Kristoffersen and Gould (28) has already been

referred to. Scarpellino and Kosikowsky (43) found methylketones and other carbonyl compounds in Cheddar. A four-carbon compound, identified by its melting point as methyl ethyl ketone, existed at relatively high concentrations in all the aged cheeses. Day and Keeney (8) stated the presence of formaldehyde, acetaldehyde, acetone, butanone, 2-pentanone, 2-nonanone, acetoin and diacetyl. They also found a sulphur-containing compound, 3-methylthiopropanol, which they considered the most important flavor component. Patton, et al. (39) reported similar results. Dimethyl sulphide, ethanol, acetone and diacetyl were conclusively identified. These authors considered dimethyl sulphide the most important flavor component.

It is worthy of noting the agreement of several investigators on the importance of sulphur-containing compounds hydrogen sulphide, dimethylsulphide and 3-methylthiopropanol which are all claimed to be of importance in Cheddar cheese flavor, possibly in combination with fatty acids.

Irvine, et al. (22) showed the importance of a moderate lipolysis in the development of cheese flavor. They inoculated pasteurized cheese milk with Geotrichum candidum eighteen hours before the manufacturing process and stated that the cheese obtained scored higher than the control and appeared more mature. The cheese contained as much C6 and higher acids as cheese made from raw milk, while the control cheese from uninoculated, pasteurized milk was almost completely free of these acids.

Patton (38) evaluated the contribution of various classes of compounds to Cheddar cheese aroma by adding reagents to selectively block functional groups and concluded that acetic, butyric, caproic and caprylic acids constitute the backbone of Cheddar cheese aroma. Because it is distinctive of Cheddar volatiles, and occurs in the highest concentrations among the volatile acids, acetic acid may be particularly significant in the aroma.

Failure to attribute Cheddar flavor to any of the major constituents of ripe cheese and the conclusion of Dacre (7) that the components of typical flavor are volatile and are present in Cheddar in concentrations of only a few p.p.m., has led investigators to examine more closely the minor volatile components of cheese, and in particular, carbonyl compounds.

Mabbitt (34), in a review of the work of various investigators on the flavor of Cheddar cheese, summarized the carbonyl compounds found in Cheddar cheese. The qualitative agreement among the different investigators is impressive. Mabbitt seems certain that the ketones, which contain odd numbers of carbon atoms, are derived by β oxidation of the C4 - C14 fatty acids of milk fat, and this view is supported by the detection of a number of appropriate keto acids (4). Examination of the flavor of suitable mixtures of ketones when incorporated into cheese curd, together with a suitable mixture of fatty acids, showed that in one case a typical cheese flavor was not produced. However, the quantitative aspect of the

analysis is unsatisfactory, and other tasting trials, in which the concentration of ketones has been varied and supplemented with a fatty acid mixture together with H_2S, have given more promising results (49).

Walker (49) added mixtures of methyl ketones and fatty acids to bland cheese curd. After three weeks, these experimental cheeses possessed a distinct cheddar-type flavor; the intensity of the artificially-induced flavor corresponding to that of a normal three-month-old Cheddar cheese. This supports the hypothesis that the normal mature Cheddar flavor results from a blend of methyl ketones, fatty acids, and single substances such as hydrogen sulphide. If this can be substantiated, the flavor problem in Cheddar cheese, from the synthetic aspect, may come from carefully controlled tasting panels.

It should be noted that evidence now suggests that the typical flavor of cheese is due to a complex mixture of components and the success of tasting trials will depend on the care given to obtain the correct component balance.

Methods of Determination of Free Acids in Cheddar Cheese

I. Direct Steam Distillation of Acidified Cheese and Titration of Distillate.

Lane and Hammer (30) steam distilled 200 g of finely-divided cheese and 400 ml of water acidified with 25 ml sulphuric acid until 1 liter of distillate was obtained. One hundred ml of the distillate were titrated with 0.1N sodium hydroxide, using phenolphthalein as indicator.

Hiscox and Harrison (16) carried out tests and showed that complete recovery of measured quantities of acid added to the cheese before distillation was rarely, if ever, attained. They claimed that one or more constituents of cheese possessed the power of retarding the rate of distillation and, in some cases, even of preventing complete recovery of the added acid.

Kosikowsky and Dahlberg (26) reported a rapid, direct-distillation procedure for determining the volatile fatty acids of cheese. A 10.0 g portion of cheese was finely ground with warm 10% sulphuric acid. The ground cheese mixture was then washed from the mortar into an 800 ml Kjeldahl flask with a total of 50 ml sulphuric acid solution, including both grinding and washing. Glass beads and 35.0 g of $MgSO_4.7H_2O$ were added to the flask. The mixture was thoroughly shaken and exactly 230 ml of distilled water were added. After 3 to 5 minute refluxing to

drive off carbon dioxide, 20 ml of distilled water were added and distillation continued until 285 ml of distillate were collected. Titration of the distillate gave the water soluble portion of the volatile acids. The insoluble acids were then rinsed from the condenser, through the filter paper, and into a small Erlenmeyer flask with neutral alcohol. Titration was carried out in a manner similar to that employed with the water distillate. The sum of the titration of the water distillate and of the alcohol rinse was considered the total volatile acidity of the cheese. For the short-chain volatile acids, such as acetic, butyric and caprylic, recovery was reported as close to 100%.

II. Solvent Extraction of Acids Followed by Distillation and Titration.

Johnson and Gould (24) reported that the free acid content of fat was higher when the fat was obtained from cream by solvent extraction than when it was obtained by churning. The free acid content of the fat was determined, in both cases, by titration of a 10.0 g sample with 0.1N NaOH to the phenolphthalein end point. They indicated that the extraction procedure possesses definite advantages over the churning method for measuring the degree of fat splitting which results from lipase activity.

Dunkley (9) stated that on the basis of acid degree determinations applied to fat obtained from cream samples, fat acidity determinations are helpful in classifying cream as "not rancid" or "rancid", but they are of little value as a measure of the intensity

of rancidity when compared with organoleptic evaluations as the standard.

Harper, et al. (14) asserted that the water soluble fatty acids are only partially recovered by solvent extraction.

Hiscox and Harrison (17) claimed that more satisfactory results were obtained when preliminary extraction of the acids from the cheese was first carried out by means of suitable solvents. Steam distillation of these solvents would not then be subject to the disturbing influence of various constituents of the cheese.

A simplified extraction-distillation method was proposed by Smiley, et al. (45). The method included the ether extraction of an acid-cheese mixture, removal of volatile fatty acids from the ether by washing with dilute alkali, and acidification of the washings, followed by direct distillation in the presence of magnesium sulphate. A specific amount of distilled water was added to the acid residual material and the mixture directly distilled with magnesium sulphate. The sum of the two distillations plus that of the alcohol rinse represented the total volatile fatty acidity of the cheese.

Barnett and Tawab (3) extracted phosphoric acid-treated cheese (pH 2.0) with ether in a Soxhlet thimble for eight hours. The ether was then extracted in a separatory funnel with sodium hydroxide solution. Following removal of the ether by evaporation on a hot plate, the concentrated residue, with aqueous washings

from the beaker, was micro-distilled and the distillate titrated.

III. Chromatography.

In 1942, Smith (46) demonstrated that silica gel partition chromatography, first developed by Martin and Synge (36), would separate, into its component acids, a mixture of formic, acetic, propionic, n-butyric and n-valeric acids in chloroform containing 1% butanol.

Peterson and Johnson (40), in an investigation of the role of fatty acids in Cheddar cheese flavor, developed a rapid method for the quantitative estimation of formic, acetic, propionic, n-butyric, caproic, caprylic and capric acids. They reported the use of benzene-aqueous sulphuric acid in partition chromatography. Fatty acids in known mixtures, or fatty acids added to butterfat samples, were recovered with a maximum error of 8%.

Brown (6) reported the separation of the lower fatty acids as anions by means of paper chromatography. The distances moved by the anions were independent of the presence of other anions. Mixtures of the sodium salts of as many as six acids gave separate spots with R_f values equal to those of the individual sodium salts run independently on the same paper.

Hiscox and Berridge (15) reported the use of paper partition chromatography for the identification of volatile fatty acids in cheese distillates. The method was based primarily on

the silica gel technique of Smith (46), in that water was used as the stationary phase, butanol as the mobile phase, and bromcresol-green as the indicator.

Hock, et al. (19) removed the acids from cheese by steam distillation and then separated them by a column chromatographic procedure, using silicic acid as the column packing and the developing solvents 3% butanol in chloroform, followed by 10% butanol in chloroform. The fractions were titrated with 0.02N alcoholic sodium hydroxide to the phenolphthalein end point. The method yielded comparative results of good accuracy for the acids acetic, propionic and butyric. The volatile acids caproic and higher were determined as a group.

Harper (11) reported a chromatographic procedure for the direct determination of acetic, propionic and butyric acids in cheese without prior distillation. He stated two principal advantages of eliminating distillation or extraction of acids from the cheese, namely, conservation of time and quantitative recovery of the insoluble volatile fatty acids. In the method reported, 20% sulphuric acid was added to 5 g of cheese to adjust the pH to between 1.7 and 2.0. Enough water was added to bring the total added volume up to 3.0 ml; 10 g of dry silicic acid were added and the mixture ground thoroughly. Fifty ml of chloroform were used to prepare a free-flowing slurry and to wash the sample into the column. A silica gel column was used

with stepwise increases in the concentration of n-butanol in chloroform as solvent. Five ml fractions were collected in Erlenmeyer flasks and titrated with 0.01N KOH in absolute ethanol, using phenol red as the indicator. The method was compared with a similar chromatographic method (19) requiring distillation prior to analysis. The values obtained with the two methods were reported as almost identical.

Harper and Armstrong (12) reported a chromatographic method for the routine measurement of butyric acid in dairy products to detect fraudulent butterfat substitution with various vegetable and/or animal fats. The method utilized the principles of both partition and adsorption chromatography and permitted the direct saponification of the fat without extracting it from the dairy product prior to determining the molar concentration of butyric acid. A buffered silicic acid column was used. The sample was hydrolyzed with potassium hydroxide and 95% ethanol prior to being placed in the column where the acids were separated using 0.75% butanol in chloroform and then 5% butanol in chloroform as the solvent.

Harper, et al. (14) modified the procedure of Harper and Armstrong (12) so that the total quantity of free fatty acids could be recovered in one fraction and with one solvent in approximately fifteen minutes. The method is reported to give a quantitative recovery of small amounts of free fatty acids from milk and cream by directly separating the acids from the product without

prior fractionation. The silica gel column was prepared in two sections, the bottom section containing a mixture of silicic acid and phosphate buffer, and the top section containing the acidified milk and silicic acid.

Wiseman and Irwin (51) reported the use of a Celite column with an internal indicator for the quantitative separation of silage acids ranging from butyric to succinic. Eluting solvents were mixtures of acetone with Skellysolve B.

Keeney (25) presented a chromatographic method for determining the molar percentage of butyric acid in fat. The method involved separating the fatty acids, derived from a fat, by partition chromatography into a pure butyric acid fraction and another fraction containing all of the other fatty acids. The two fractions were titrated with alkali and the mole percent butyric acid calculated from the sum of the two titrations. The procedure called for saponification of the fat and the obtaining of the fatty acids in an hexane-butanol solution. The fatty acid solution was then eluted from a silicic acid column, using hexane-butanol as solvent. The long-chain fatty acids, C6 and higher, passed rapidly through the column. The yellow butyric acid zone was easily noted and the fraction collected.

The advent of gas-liquid chromatography made it possible to separate the compounds of an homologous series more readily.

Hankinson, et al. (10) developed a gas-liquid chromatographic (GLC) method for evaluating individual volatile free fatty

acids in milk. Formic through caprylic acids were determined by titration after separation on the GLC column and collection in distilled water.

Many investigators have resorted to the use of methyl esters rather than free fatty acids for GLC separation. Hornstein, et al. (21) reported a method for the isolation, conversion to methyl esters, and GLC analysis of free fatty acids occurring in the presence of large amounts of unsaponified fat. The fatty acids were adsorbed on a strong anion base exchange resin, then washed free of fat with petroleum ether and the free fatty acids converted directly to their methyl esters on the resin with HCl-methanol. This method was applied to the free fatty acids of cooked and cured meats which essentially contained lauric through linolenic acids.

Milk fat presents the problem of handling the more volatile short-chain acids or their methyl esters.

Bills, et al. (5) isolated the free fatty acids of milk from the fat by means of a basic anion exchange resin, converted them to methyl esters, and extracted these with ethyl chloride. The ethyl chloride solution of methyl esters was then concentrated prior to chromatography with a special reflux system to prevent the loss of the more volatile esters. Appropriate factors were calculated for relating the quantity of added internal standards to that of the naturally occurring free fatty acids.

A low-temperature, high-vacuum distillation technique, utilizing a molecular still, was used by Libbey, et al. (32) in studying food flavor volatiles. Identification of the flavor volatiles was based on relative specific retention volume and collection of the fractions for analysis by techniques such as mass spectrometry.

EXPERIMENTAL METHODS

I. Source of Cheese Samples.

(i) Mild, Medium and Old Cheddar cheeses were obtained from local retail stores. The samples were taken directly from the counter and would represent a sampling of the cheeses normally purchased by the consumer. No identification, as to date or particulars of manufacture, was available. It should be pointed out that no definite grade standards for Cheddar cheese are used in the Canadian retail trade.

(ii) Samples of Alberta Cheddar cheese which were graded by official government graders as "unclean" or "rancid" at the time of grading were procured and these were identifiable as to date and place of manufacture. Samples were divided into two separate lots and individually waxed, one lot being stored in a cold room (40° - 45° F) and the other in a freezer (-6° to -10° F). The low-temperature storage was used to prevent further acid development pending analysis.

(iii) Particulars of manufacture were available on samples of cheese made in the Department of Dairy and Food Science, University of Alberta, Edmonton and these were waxed and stored in the cold room pending analysis.

(iv) One sample each of Gouda, Edam and Swiss, and two samples of commercial Alberta Cheddar pasteurized-milk cheese were

obtained from local retail stores, no identification as to date and particulars of manufacture being available.

II. Procedure of Analysis.

(i) A modified Babcock test was used to determine the fat content of the cheese. A 9.0 g sample was weighed into a 50% cream test bottle and 12 ml of water, at a temperature between 71° C and 76° C, added and thoroughly mixed. A total of 17.5 ml of sulphuric acid was then added in four separate additions with shaking between each addition. The sample was centrifuged and tempered as for the standard Babcock test. Reader oil was used to eliminate the meniscus. All samples were run in duplicate.

(ii) Fat from the cheese for the titration of acidity was obtained by centrifugation. Approximately 90 g of cheese were placed in a Servall SS-3 super-speed centrifuge operating at 30,000 x g for 60 minutes. As the rotor of the centrifuge operates at 12° - 15° C above room temperature, the cheese fat was liquified during centrifugation and was easily decanted. Recovery of approximately 15 g of fat was realized by this method. This represented a recovery of almost 50% of the fat present in the cheese.

(iii) Five g of cheese fat in a 50 ml Erlenmeyer flask were titrated with 0.05N alcoholic potassium hydroxide to determine the fat acidity. The acidity of milk fat is usually expressed as acid degree value, that is, milliliters of 1N NaOH required to

neutralize 100 g of fat. By titrating a 5.0 g cheese fat sample with 0.05N potassium hydroxide, the results can be expressed in acid degree value which is the same as mmoles of acid per 100 g of fat. The fat was titrated in a solvent mixture consisting of 10 ml of neutral methanol and 10 ml of petroleum ether and 5 drops of phenolphthalein as an indicator. The petroleum ether assisted in "sharpening" the end point reading. The use of a magnetic stirrer aided in the titration.

(iv) The steam volatile acids in 5.0 g of cheese were distilled using equipment specified for the Reichert Meissl determination. The laboratory set-up consisted of a battery of six distillation units with ice-water cooling of the condensers. This arrangement permitted duplicate distillations on three samples to be carried out simultaneously. The 5.0 g samples of cheese were weighed and placed in a 300 ml round-bottom flask, to which was added 140 ml of distilled water, 25 ml of 10% sulphuric acid, 20 g $MgSO_4.7H_2O$, some boiling stones and a drop of antifoam. The sulphuric acid was added to acidify the cheese "mush" and liberate the volatile acids from their salts. The $MgSO_4.7H_2O$ was used to raise the boiling point.

The distillation was carried out until 150 ml of distillate were collected in a 300 ml Erlenmeyer flask, the rate of distillation being regulated to obtain this amount in approximately 30 minutes. To ensure that all of the steam volatile water soluble acids that

were distilled over were being collected in the Erlenmeyer flask, the tip of the condenser was extended to the bottom of the flask so that the tip was completely covered by the distillate. Several distillations proved, however, that this was not necessary and it was discontinued. The condenser and connecting glass tubing were rinsed with neutral alcohol to dissolve the water insoluble fatty acids. A number of distillations were carried out using all of the above materials except the cheese to determine the blank titration.

(v) The cheese distillates were titrated with 0.05N alcoholic potassium hydroxide, using phenolphthalein as an indicator. The blank titration showed an average acidity of 0.2 ml and this amount of acidity was deducted from all the cheese distillations. As explained before in connection with the cheese fat titration, the use of a 5.0 g cheese sample and 0.05N alcoholic potassium hydroxide as titrant gives the acid degree and the number of ml of titrant is equivalent to the milliequivalents of volatile acid per 100 g of cheese.

(vi) The volatile acid distillates were evaporated to dryness leaving the potassium salts of the acids. The salts were then redissolved in 2 ml of distilled water and transferred to an especially-designed test tube (Fig. 1) and evaporated to dryness again.

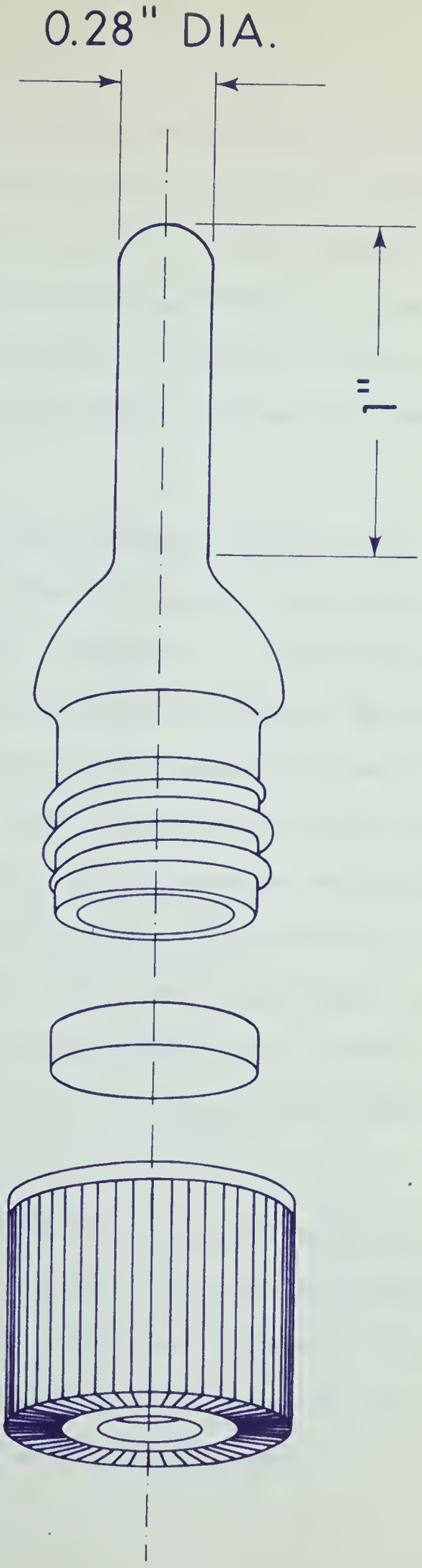

Fig. 1 Special test tube for releasing volatile acids from the salts.

(vii) The acids were released from the salts by adding 0.2 ml of a 10% potassium bisulfate solution to the test tube which was immediately recapped. To ensure complete liberation of the acids, the test tube was agitated for 2 minutes on a Vortex mixer. The acids from acetic to caproic are water soluble and therefore these acids were readily available for sampling with a microliter syringe.

(viii) Gas-chromatographic analysis of the acids in the aqueous solution was conducted using a Wilkens Hi-Fi flame ionization gas-chromatograph. By means of a microliter syringe, 0.5 microliters of solution were obtained through the rubber septum of the special test tube and introduced into the GLC column. The column used was a 5' x 1/8" in diameter, stainless steel coil, packed with 20% neopentyl glycol succinate on 60/80 mesh Firebrick treated with 2% phosphoric acid. The instrument was temperature programmed from 130° C - 150° C and operated with a nitrogen flow of 25 ml/min. Duplicate chromatograms were obtained for the acids up to and including caproic. A typical chromatogram is shown in Fig. 2.

A model 44 Photovolt Microcord recorder was used to chart the gas-chromatograph response. To obtain chromatograms that were readily measurable, the recorder was operated at 2" per minute until butyric acid was eluted; it was then switched to 1" per minute for the balance of the chromatogram.

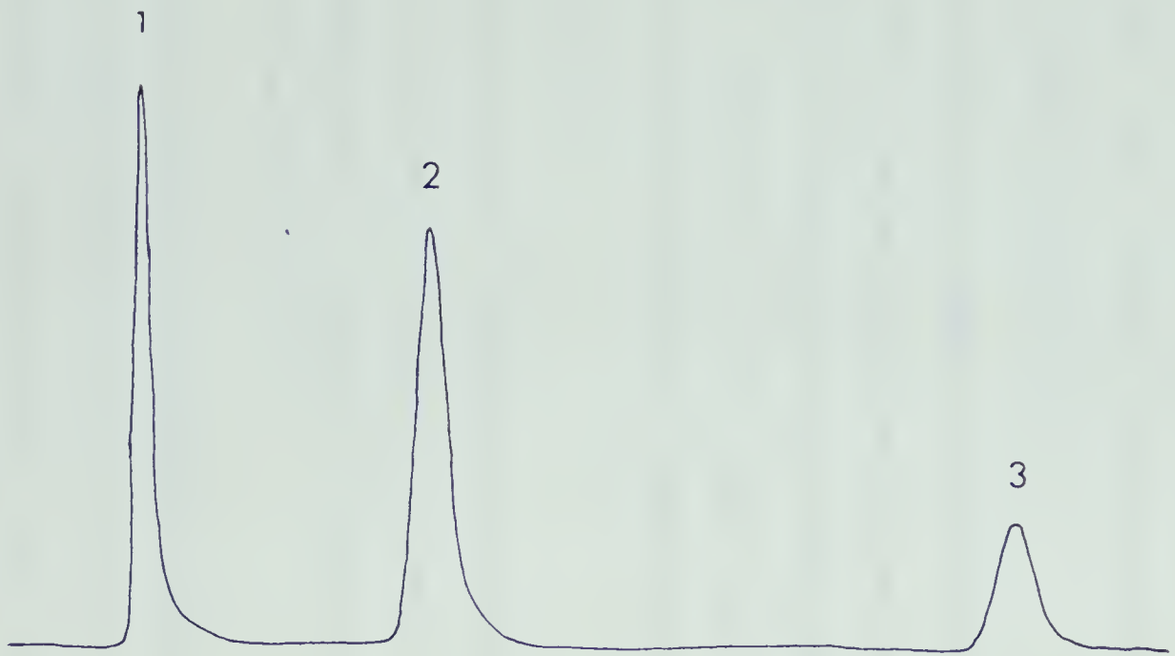

Fig. 2 Gas-chromatogram of volatile acids from sample ME - 4 (aqueous portion): peak 1 - acetic acid, peak 2 - butyric acid, peak 3 - caproic acid.

(ix) After determination of the water soluble acids, 0.2 ml of carbon disulfide were added to the test tube, the contents were again agitated with a vortex mixer and 1.0 microliter of the carbon disulfide layer was withdrawn with a microliter syringe and injected into the GLC column. The instrument was operated isothermally at 190° C and duplicate chromatograms obtained. A typical chromatogram is shown in Fig. 3.

Preliminary experiments had shown that the carbon disulfide extraction of the potassium bisulfate solution resulted in quantitative transfer of all acids with six or more carbon atoms to the organic layer. Acids with up to six carbon atoms were soluble in the potassium bisulfate solution but those with eight or more carbon atoms were not.

Identification of the peaks on the chromatograms was made by comparison of the retention times with those of authentic compounds. The quantitative evaluation of peak areas was made by measurement with an Ott-planimeter.

The two-step gas-chromatographic analysis yielded quantitative caproic peaks on both the aqueous solution chromatogram and the carbon disulfide layer chromatogram. The ratio of areas of all acids was then readily obtained by multiplying all the other peaks on the carbon disulfide layer chromatogram by a ratio of:

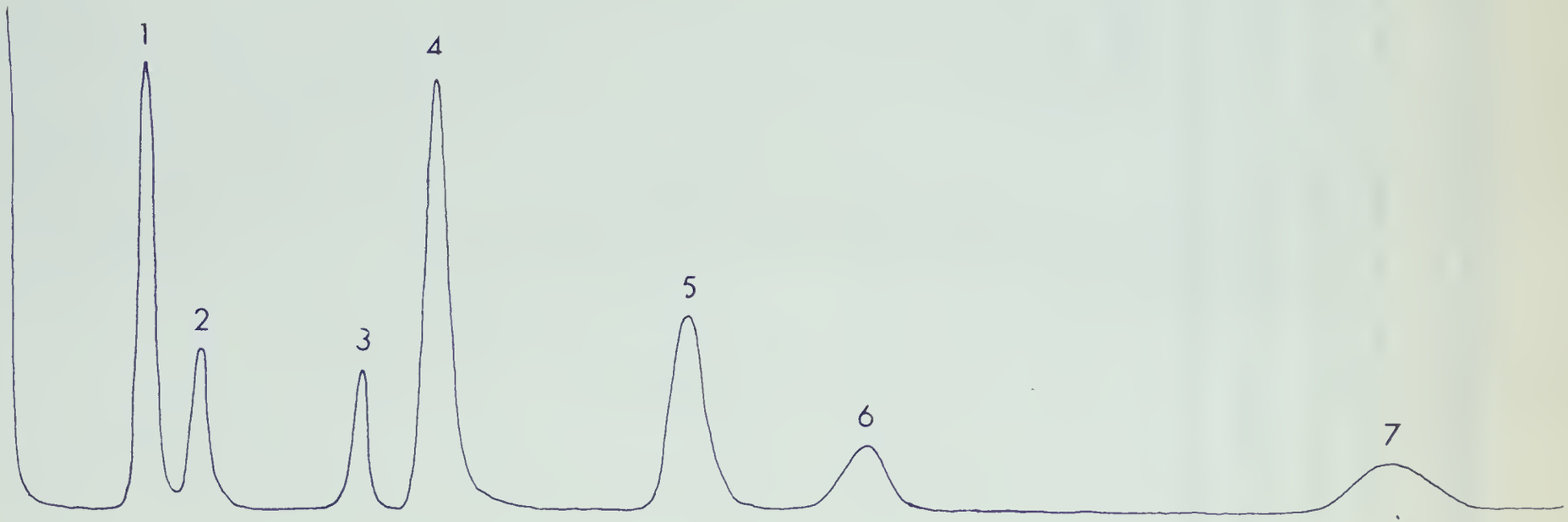

Fig. 3 Gas-chromatogram of volatile acids from sample ME - 6 (CS_2 portion): peak 1 - caproic acid, peak 2 - unknown, peak 3 - caprylic acid, peak 4 - unknown A, peak 5 - capric acid, peak 6 - unknown B, peak 7 - lauric acid.

$$\frac{\text{caproic peak area on aqueous solution chromatogram}}{\text{caproic peak area on carbon disulfide layer chromatogram}}$$

By dividing the ratio of areas of the acids with the molecular weights of each acid, the molar ratios of the acids were obtained; and knowing the milliequivalents of the volatile acid per 100 g of cheese from the titration, the milliequivalents of each acid per 100 g of cheese were then calculated.

RESULTS

I. Mild, Medium and Old Cheddar Cheeses.

Tables 1, 2, 3 and 4 show the results of the butterfat test, fat acidity and volatile acidity on the 15 mild (M), 14 medium (ME) and 14 old (O) samples of cheese.

(i) Butterfat. The butterfat content of all samples was within a very small range of values. The mild samples were within a range of 33.5% to 35.5% with a mean of 34.3%, the medium 33.0% to 38.5% with a mean of 35.2% and the old 32.5% to 36.0% with a mean of 34.8%.

(ii) Fat acidity. The fat acidity, expressed in mmoles per 100 g fat, showed a considerable range in values. In the mild cheeses there was a range of from 1.03 to 4.06 with a mean of 2.50, in the medium cheeses from 1.03 to 6.17 with a mean of 2.51 and in the old cheeses from 1.74 to 5.46 with a mean of 3.37.

(iii) Volatile acids. The volatile acids, expressed in mmoles per 100 g cheese, indicated quite a range in values; the mild from 0.94 to 2.88 with a mean of 1.87, the medium from 1.25 to 3.40 with a mean of 2.12 and the old from 1.32 to 4.19 with a mean of 2.48.

As reported, under procedure of analysis, the gas-chromatographic analyses were performed in two steps, the first one in aqueous solution for the acids from acetic to caproic, the second in carbon disulfide

for the acids from caproic to capric. As caproic acid was present on both gas-chromatograms, the amounts of all acids could be calculated. It had previously been determined that when the carbon disulfide was added to the aqueous solution, all of the caproic acid was transferred to the carbon disulfide layer.

Figs. 2, 3, 4 and 5 show typical gas-chromatograms obtained from medium and old cheeses. The volatile acids present in the aqueous solution and the carbon disulfide layer of ME - 4 and ME - 6, respectively, are shown in Figs. 2 and 3; while Figs. 4 and 5 show the volatile acids in the aqueous solution of 0 - 10 and 0 - 5.

The distribution of the volatile acids in the mild, medium and old cheeses is presented on a mole % basis in Tables 5, 6, 7 and 8 and on a mg/kg of cheese basis in Tables 9, 10, 11 and 12. The mole % of the acids was calculated by dividing the peak area ratio of each of the volatile acids by their molecular weight to obtain the molar ratio of the acids. From the molar ratio, the mole % was calculated and, from the previously determined acidity, the amount of each acid in mg/kg of cheese was then calculated.

Two unknown peaks appeared on the chromatograms of all but six of the mild, medium and old cheeses (see Figs. 3 and 6). Unknown A appeared between caprylic and capric and, for purposes of calculation of the molar ratio, was assigned a molecular weight midway between caprylic and capric (158.2). Unknown B appeared between capric and lauric and for calculation of molar ratio was

assigned a molecular weight between capric and lauric (186.3). From Tables 5, 6 and 7, it will be seen that Unknown A was present in 10 of the 43 samples and Unknown B in 8, in amounts greater than 1.0 mole %. The highest amount for any one sample of these unknowns was 15.0% for A and 1.8% for B, the mean for all samples being 2.1% and 0.7%, respectively. A search of the literature failed to reveal any reports of volatile acids in the regions where Unknown A and Unknown B were located. Further investigation of these two unknown acids would be desirable.

Referring again to Tables 5, 6 and 7, one observes that the mole % acidity of the volatile acids present in each of the three classes of cheeses had a wide range. For mild cheeses, acetic acid showed a range of from 69.7% to 94.8%, the butyric acid from 3.5% to 12.8% and the caproic acid from 0.8% to 3.8%. The ranges in the medium cheeses were: acetic from 66.5% to 88.7%, butyric from 5.1% to 21.1% and caproic from 1.9% to 3.9%. Similarly for the old cheeses the figures were: acetic acid 42.4% to 86.1%, butyric acid 6.9% to 39.0% and caproic acid 2.0% to 7.4%.

Table 8 summarizes the distribution on a molar % basis of the volatile acids. The acetic acid level was highest in the mild cheeses with a mean of 82.5%, medium and old cheeses having a mean of 79.1% and 73.3%, respectively. There was an increase in the butyric acid level from a mean of 8.8% to 11.6% to 15.3% for these three groups of cheeses and the caproic acid level also increased from 2.1% to 2.9% and to 3.9%. The figures for caprylic

acid were 1.1%, 1.3% and 1.6%.

The amounts of volatile acids in mg/kg of cheese are given in Tables 9, 10 and 11. The acetic acid in the mild samples had a range of 429 to 1281; butyric, 78 to 202; caproic, 28 to 62; caprylic 9 to 36, and capric, 18 to 93. The figures for the medium samples were: acetic, 588 to 1610; butyric, 79 to 497; caproic, 39 to 112; caprylic, 19 to 81 and capric, 27 to 141. The range for the old samples showed acetic to be from 336 to 1509, butyric from 118 to 1439, caproic from 48 to 304, caprylic from 26 to 105 and capric from 43 to 152.

Table 12 gives the mean, in mg/kg of cheese, of the volatile acids found in the samples analyzed. There was only a slight increase from mild to old in the amount of acetic acid but there was a substantial increase in the amounts of butyric and caproic acids. The mean of butyric acid in the mild cheeses was 136, in the medium 218, and in the old 350. For caproic acid the relative values were 42, 71 and 113. Propionic acid was found in M - 13, M - 14, M - 15, ME - 5 and in O - 5. In all five cases, the amount was sufficiently high to be included in the calculation of the molar ratio of acids.

A small unknown peak appeared on the chromatograms of two samples ME - 6 and O - 8, positioned between caproic and caprylic acids. The unknown peak on the chromatogram of ME - 6 is shown in Fig. 3. In the case of ME - 6, it represented only 0.9 mole % of the volatile acidity and 29 mg/kg of cheese. In regard

to 0 - 8, the peak was quite small and was not calculated.

Lauric acid was found in 7 out of the 15 mild cheese samples, in 6 out of the 14 medium and in 8 out of the 14 old samples. One would not expect to quantitatively recover lauric acid by the steam distillation method used to obtain the volatile acids. For this reason no mean is shown for lauric acid in the tables. Where lauric acid was found in the samples however, it has been shown in the tables and used in calculating the molar ratio of acids.

II. Unclean and Rancid Alberta Cheddar Cheese.

Table 13 shows the results of the butterfat test, fat acidity and volatile acid analyses on 7 unclean (U) and 3 rancid (R) samples of cheese analyzed. The gas-chromatograms obtained from the volatile acids present in the aqueous and carbon disulfide layer of R - 1 are shown in Figs. 7 and 8, respectively. Samples U - 1, U - 2, U - 3, U - 4 and U - 5 were held for 240 days in the freezer from date of manufacture until analyses were conducted. Samples U - 6 and U - 7 were held in the cold room for 441 days and 207 days, respectively, from date of manufacture until analyses were conducted. This same procedure was followed for the rancid samples, R - 1 and R - 2 being held in the freezer for 217 and 327 days, respectively, and R - 3 in the cold room for 411 days.

(i) Butterfat. There was only a small range in values for butterfat content. The unclean samples ranged from 34.0% to

to 35.5% with a mean of 35.0%. Two of the rancid samples tested 35.0%, the other one 36.5%.

(ii) Fat acidity. There was very little difference in fat acidity of the unclean samples. The acidity ranged from 1.27 to 1.91 mmoles/100 g fat, with a mean of 1.53. The rancid samples showed a marked difference, R - 1 containing 2.29, R - 2, 1.32 and R - 3, 1.50 mmoles/100 g fat. An increase in fat acidity in the unclean samples took place under conditions of cold room storage. These samples were held in both the cold room and freezer for 240 days from date of manufacture until analyzed. Table 14 shows the increase in fat acidity in the samples held in the cold room over the fat acidity of those in the freezer.

(iii) Volatile acids. A considerable range in the quantity of volatile acids was evident in both the unclean and rancid cheeses (Table 13). Unclean samples ranged from 1.51 to 3.31 mmoles/100 g cheese, with a mean of 2.36; those that were rancid had values of 0.71, 1.46 and 2.09 mmoles/100 g cheese.

The results of gas-chromatographic analyses of volatile acids are shown for these cheeses in Table 15 which gives the molar distribution and it will be noted from the table that Unknowns A and B were present in all the samples of both the unclean and rancid cheeses. In the unclean samples, acetic acid ranged from 72.4% to 92.6% with a mean of 87.2%, butyric acid from 3.3% to 19.3% with a mean of 7.4% and caproic acid from 1.0% to 3.3% with

a mean of 1.8%. Mean contents of capric and lauric acid were 0.9% and 1.2%, respectively. The acetic acid levels were much lower and the butyric and caproic acids much higher in the rancid samples. The mean of acetic acid was 63.2%, for butyric 27.8%, for caproic 4.2%, for caprylic 1.4% and for capric 1.8%.

Table 16 shows the amount of volatile acids in mg/kg of cheese. Acetic acid, in the unclean samples, showed a range of from 789 to 1824, butyric from 65 to 433, caproic from 27 to 97, caprylic from 22 to 49 and capric from 21 to 89. The mean for acetic was 1234, butyric 159, caproic 49, caprylic 29 and capric 49. In the rancid samples relatively high levels of butyric acid were associated with relatively low levels of acetic acid. The butyric acid mean was 350 while the acetic mean was only 544. The mean for caproic acid was 65, for caprylic 25 and for capric 40.

Table 17 shows the increase in the volatile acids of the unclean samples under cold room as compared to freezer storage.

III. University of Alberta (UA) Cheddar Cheese.

The results of the fat test, fat acidity and volatile acid analyses on five lots of cheese made in the Department of Dairy and Food Science at the University of Alberta, Edmonton are shown in Table 18. Lots UA - 1 and UA - 2 were made from the same shipment of milk, with UA - 2 having 10% raw milk added. Similarly

lots UA - 3 and UA - 4 were of the same shipment with UA - 4 having 10% raw milk added. Samples were analyzed for fat acidity at approximately 1 month and 5 to 6 months' of age. Volatile acids were determined at about 1 month, 3 months and 5 to 6 months' of age. Lots were held in the cold room until analyzed. In the one-month old cheese both the fat acidity and volatile acids were very low. There was a slow increase in both fat acidity and volatile acids during the 5 to 6 months' period. The fat acidity, however, was still much lower and the volatile acidity approximately the same as for the mild samples reported previously.

Gas-chromatograms of the volatile acids present in the aqueous solution of UA - 1 and UA - 2 are shown in Figs. 9 and 10.

Distribution of the volatile acids on the basis of mole % and mg/kg of cheese is given in Tables 19 and 20, respectively. High levels of acetic and low levels of butyric, caproic and caprylic acid were found. The acetic, butyric and caproic acid levels in the 5 to 6 months'-old cheese were appreciably lower than in the mild cheeses examined (Tables 5 and 9).

IV. Gouda, Edam, Swiss and Alberta Pasteurized Cheddar Cheeses.

Table 21 shows the results of the butterfat test, fat acidity and volatile acid analyses of one sample of each of Gouda, Edam and Swiss cheese and on two samples of commercial Alberta pasteurized-milk Cheddar cheese. The very high volatile acidity

and low butterfat test of the Swiss sample of cheese is noticeable. The two Alberta pasteurized-milk Cheddar cheese samples show a wide range in both fat acidity and volatile acidity, particularly in the latter.

TABLE 1.

Fat Test, Fat Acidity and Volatile Acids in Mild (M) Cheddar Cheese

Sample	Fat Test %	Fat Acidity mmoles per 100 g fat	Volatile Acids mmoles per 100 g cheese
M - 1	34.0	3.11	2.04
M - 2	35.0	1.58	2.10
M - 3	35.5	1.60	0.94
M - 4	34.0	1.70	1.99
M - 5	35.5	1.03	2.88
M - 6	33.5	2.50	1.87
M - 7	34.0	2.32	1.95
M - 8	33.5	3.23	2.31
M - 9	34.5	2.53	1.87
M - 10	34.0	1.95	2.39
M - 11	34.0	3.26	1.97
M - 12	35.5	3.36	1.79
M - 13	34.0	4.06	1.15
M - 14	34.5	3.11	1.22
M - 15	34.0	2.20	1.52
Mean	34.3	2.50	1.87

TABLE 2

Fat Test, Fat Acidity and Volatile Acids in Medium (ME) Cheddar Cheese

Sample	Fat Test %	Fat Acidity mmoles per 100 g fat	Volatile Acids mmoles per 100 g cheese
ME - 1	33.0	2.41	1.68
ME - 2	34.0	2.02	2.13
ME - 3	36.0	1.03	1.90
ME - 4	38.5	2.48	1.58
ME - 5	34.5	2.93	3.40
ME - 6	35.5	3.05	2.42
ME - 7	34.0	1.95	2.16
ME - 8	35.5	1.60	1.76
ME - 9	35.5	2.69	1.25
ME - 10	35.0	6.17	2.67
ME - 11	36.0	2.43	1.41
ME - 12	34.0	2.21	2.60
ME - 13	36.0	1.66	1.65
ME - 14	36.0	2.59	3.02
Mean	35.2	2.51	2.12

TABLE 3.

Fat Test, Fat Acidity and Volatile Acids in Old (O) Cheddar Cheese

Sample	Fat Test %	Fat Acidity mmoles per 100 g fat	Volatile Acids mmoles per 100 g cheese
O - 1	36.0	3.58	1.89
O - 2	35.0	3.60	3.10
O - 3	36.0	3.01	2.78
O - 4	35.0	3.87	2.26
O - 5	34.0	3.10	3.34
O - 6	35.5	5.46	2.31
O - 7	33.5	4.86	1.32
O - 8	36.0	1.88	1.95
O - 9	35.5	2.36	1.77
O - 10	33.5	3.99	3.00
O - 11	34.5	1.74	2.43
O - 12	32.5	2.21	2.11
O - 13	35.5	3.33	2.22
O - 14	35.0	4.21	4.19
Mean	34.8	3.37	2.48

TABLE 4.

Fat Test, Fat Acidity and Volatile Acids
in Mild (M), Medium (ME) and Old (O) Cheddar Cheese

	No. of Samples	Fat Test %	Fat Acidity mmoles per 100 g fat	Volatile Acids mmoles per 100 g cheese
Mean of (M) Samples	15	34.3	2.50	1.87
Mean of (ME) Samples	14	35.2	2.51	2.12
Mean of (O) Samples	14	34.8	3.37	2.48
Mean of all Samples	43	34.8	2.79	2.16

TABLE 5.

Volatile Acids in Mild (M) Cheddar Cheese in Mole %

Sample	Volatile Acids								
	C2	C3	C4	C6	C8	C10	C12	Unknown A	Unknown B
M - 1	69.7	-	10.9	1.2	0.9	1.2	0.8	15.0	0.3
M - 2	86.7	-	7.0	1.4	0.8	1.3	1.4	0.6	0.9
M - 3	79.5	-	9.5	3.8	2.0	2.8	-	0.6	1.8
M - 4	85.6	-	8.5	2.5	0.9	0.9	-	0.3	1.3
M - 5	94.8	-	3.5	0.8	0.2	0.4	-	-	0.3
M - 6	87.0	-	7.7	2.7	1.2	0.8	-	-	0.8
M - 7	84.8	-	7.2	2.2	1.3	2.8	0.6	0.5	0.7
M - 8	88.8	-	5.2	1.7	1.1	1.3	0.6	0.6	0.9
M - 9	85.8	-	8.2	1.7	1.2	1.8	0.4	0.5	0.4
M - 10	89.3	-	6.5	1.3	0.7	0.8	0.6	0.5	0.2
M - 11	80.5	-	8.9	1.5	0.8	1.2	0.3	6.4	0.4
M - 12	80.7	-	12.8	2.9	1.3	1.1	-	0.4	0.8
M - 13	78.7	3.6	13.2	2.1	1.3	1.2	-	-	-
M - 14	72.6	7.8	9.8	2.2	1.6	2.2	-	3.7	0.2
M - 15	73.3	7.3	13.3	3.5	1.1	0.9	-	0.4	0.2
Mean	82.5	-	8.8	2.1	1.1	1.4	-	2.0	0.6

TABLE 6.

Volatile Acids in Medium (ME) Cheddar Cheese
in Mole %

Sample	Volatile Acids								
	C2	C3	C4	C6	C8	C10	C12	Unknown A	Unknown B
ME - 1	74.8	-	12.7	3.8	1.2	7.5	-	-	-
ME - 2	86.1	-	9.0	2.3	0.6	0.9	-	0.2	0.8
ME - 3	76.9	-	16.8	3.9	1.2	0.9	-	-	0.4
ME - 4	76.2	-	15.8	3.9	1.4	1.7	-	0.4	0.7
ME - 5	78.9	3.1	10.2	2.8	1.7	2.4	-	0.1	0.8
ME - 6	68.6	-	11.0	2.6	1.5	1.8	0.6	12.3	0.7
ME - 7	82.6	-	9.3	2.6	1.2	1.5	0.9	0.7	1.2
ME - 8	88.7	-	5.1	1.9	1.1	1.8	0.4	0.4	0.5
ME - 9	78.4	-	10.1	3.6	2.2	3.2	-	1:2	1.3
ME - 10	66.5	-	21.1	3.1	1.6	1.6	0.7	5.1	0.4
ME - 11	84.1	-	8.4	2.4	1.5	1.8	0.5	0.7	0.7
ME - 12	87.1	-	7.7	2.0	0.8	0.9	0.3	0.7	0.6
ME - 13	78.9	-	14.8	3.5	1.1	0.9	-	0.2	0.6
ME - 14	79.1	-	10.8	2.8	1.2	1.1	-	3.9	1.1
Mean	79.1	-	11.6	2.9	1.3	2.0	-	1.9	0.7

TABLE 7.

Volatile Acids in Old (O) Cheddar Cheese
in Mole %

Sample	Volatile Acids								
	C2	C3	C4	C6	C8	C10	C12	Unknown A	Unknown B
O - 1	77.2	-	13.4	3.7	1.8	2.0	1.0	0.3	0.7
O - 2	72.1	-	10.9	3.1	1.1	1.1	-	10.9	0.8
O - 3	81.0	-	11.7	4.0	1.5	1.8	-	-	-
O - 4	81.2	-	9.6	4.9	1.8	1.2	-	0.3	1.0
O - 5	70.8	9.4	13.5	3.8	1.0	0.7	-	0.2	0.7
O - 6	65.5	-	22.2	6.0	2.6	2.6	-	0.3	0.9
O - 7	42.4	-	30.9	7.4	3.6	4.5	0.6	9.6	1.0
O - 8	86.1	-	6.9	2.1	1.3	1.4	0.5	0.4	1.3
O - 9	81.9	-	10.2	2.5	1.5	2.0	0.3	0.3	1.4
O - 10	83.8	-	10.4	2.7	1.0	1.6	0.3	-	0.3
O - 11	85.7	-	6.7	2.2	1.6	2.3	-	0.4	1.2
O - 12	75.9	-	9.9	2.0	0.9	1.4	0.5	8.6	0.8
O - 13	72.4	-	19.2	4.2	1.6	1.9	0.2	0.2	0.3
O - 14	50.3	-	39.0	6.2	1.7	2.1	0.4	0.1	0.2
Mean	73.3	-	15.3	3.9	1.6	1.9	-	2.3	0.8

TABLE 8.

Volatile Acids in Mild (M), Medium (ME) and Old (O) Cheddar Cheese in Mole %

	No. of Samples	Volatile Acids						
		C2	C4	C6	C8	C10	Unknown A	Unknown B
Mean of (M) Samples	15	82.5	8.8	2.1	1.1	1.4	2.0	0.6
Mean of (ME) Samples	14	79.1	11.6	2.9	1.3	2.0	1.9	0.7
Mean of (O) Samples	14	73.3	15.3	3.9	1.6	1.9	2.3	0.8
Mean of all Samples	43	78.3	11.9	3.0	1.3	1.8	2.1	0.7

TABLE 9.

Volatile Acids in Mild (M) Cheddar Cheese
in mg/kg Cheese

Sample	Volatile Acids								
	C2	C3	C4	C6	C8	C10	C12	Unknown A	Unknown B
M - 1	853	-	195	29	26	43	32	484	13
M - 2	1092	-	128	34	23	46	59	21	36
M - 3	429	-	78	42	28	45	-	9	32
M - 4	1022	-	148	58	25	31	-	10	49
M - 5	1638	-	89	28	9	18	-	-	16
M - 6	976	-	126	59	31	24	-	-	26
M - 7	993	-	124	51	36	93	22	15	24
M - 8	1231	-	105	45	35	51	29	20	37
M - 9	963	-	134	37	33	56	16	15	15
M - 10	1281	-	137	37	24	33	30	19	8
M - 11	952	-	155	34	24	40	10	199	15
M - 12	867	-	202	60	33	34	-	12	28
M - 13	543	30	134	28	22	24	-	-	-
M - 14	531	71	106	31	28	47	-	71	4
M - 15	669	82	178	62	25	23	-	9	7
Mean	936	-	136	42	27	40	-	59	21

TABLE 10.

Volatile Acids in Medium (ME) Cheddar Cheese in mg/kg Cheese

Sample	Volatile Acids								
	C2	C3	C4	C6	C8	C10	C12	Unknown A	Unknown B
ME - 1	754	-	187	74	30	218	-	-	-
ME - 2	1100	-	169	58	19	34	-	8	33
ME - 3	877	-	281	86	32	28	-	-	15
ME - 4	722	-	220	71	32	47	-	9	21
ME - 5	1610	78	306	112	81	141	-	5	50
ME - 6	996	-	235	73	51	76	31	470	33
ME - 7	1071	-	177	66	38	57	37	23	50
ME - 8	937	-	79	39	29	54	14	11	17
ME - 9	588	-	111	52	39	69	-	25	30
ME - 10	1065	-	497	97	60	73	37	214	18
ME - 11	711	-	104	39	30	44	15	17	17
ME - 12	1359	-	176	60	30	39	16	30	27
ME - 13	781	-	214	67	25	27	-	6	18
ME - 14	1433	-	287	99	54	58	-	186	61
Mean	1000	-	217	71	39	69	-	72	28

TABLE 11.

Volatile Acids in Old (O) Cheddar Cheese
in mg/kg Cheese

Sample	Volatile Acids								
	C2	C3	C4	C6	C8	C10	C12	Unknown A	Unknown B
O - 1	875	-	223	80	50	64	37	9	25
O - 2	1341	-	298	113	51	56	-	535	44
O - 3	1351	-	287	129	60	87	-	-	-
O - 4	1101	-	192	128	57	47	-	10	43
O - 5	1418	233	396	146	46	43	-	9	44
O - 6	907	-	451	161	86	105	-	10	39
O - 7	336	-	359	113	69	102	16	201	25
O - 8	1007	-	118	48	36	47	20	13	49
O - 9	870	-	159	51	37	61	9	9	46
O - 10	1509	-	274	92	44	81	17	1	16
O - 11	1250	-	144	62	55	95	-	14	52
O - 12	960	-	185	49	26	52	22	288	31
O - 13	964	-	375	108	52	73	7	8	12
O - 14	1264	-	1439	304	105	152	30	5	17
Mean	1082	-	350	113	55	76	-	79	32

TABLE 12.

Volatile Acids in Mild (M), Medium (ME) and Old (O) Cheddar Cheese in mg/kg Cheese

	No. of Samples	Volatile Acids						
		C2	C4	C6	C8	C10	Unknown A	Unknown B
Mean of (M) Samples	15	936	136	42	27	40	59	21
Mean of (ME) Samples	14	1000	218	71	39	69	72	28
Mean of (O) Samples	14	1082	350	113	55	76	79	32
Mean of all Samples	43	1005	232	75	40	61	70	27

TABLE 13.

Fat Test, Fat Acidity and Volatile Acids
in Unclean (U) and Rancid (R) Cheddar Cheese

Sample	Fat Test %	Fat Acidity mmoles per 100 g fat	Volatile Acids mmoles per 100 g cheese
U - 1	35.0	1.45	2.26
U - 2	34.5	1.47	2.26
U - 3	35.5	1.40	1.51
U - 4	34.0	1.91	2.55
U - 5	34.5	1.27	1.97
*U - 6	36.0	1.70	2.67
*U - 7	35.5	1.50	3.31
Mean	35.0	1.53	2.36
R - 1	35.0	2.29	2.09
R - 2	35.0	1.32	0.71
*R - 3	36.5	1.50	1.46
Mean	35.5	1.70	1.42

*Stored in cold room at 40° F - other samples in freezer at -10° F.

TABLE 14.

Fat Acidity in Unclean (U) Cheddar Cheese Stored in Cold Room and Freezer

Fat Acidity in mmoles/100 g fat

Sample	Freezer	Cold Room
U - 1	1.45	1.92
U - 2	1.47	1.86
U - 3	1.40	1.87
U - 4	1.90	1.43
Mean	1.56	1.77

TABLE 15.

Volatile Acids in Unclean (U) and Rancid (R) Cheddar Cheese in Mole %

Sample	Volatile Acids							
	C2	C4	C6	C8	C10	C12	Unknown A	Unknown B
U - 1	92.6	3.3	1.0	0.7	0.9	0.3	0.4	0.9
U - 2	92.3	3.7	1.1	0.7	1.0	0.2	0.3	0.8
U - 3	87.1	5.7	1.9	1.0	0.9	-	0.5	2.9
U - 4	72.4	19.3	3.3	1.3	2.0	0.7	0.3	0.7
U - 5	90.9	4.1	1.4	0.9	1.1	0.3	0.3	1.1
*U - 6	83.3	11.0	2.4	0.9	1.4	0.5	0.3	0.3
*U - 7	91.8	4.6	1.3	0.6	1.0	0.3	0.1	0.3
Mean	87.2	7.4	1.8	0.9	1.2	-	0.3	1.0
R - 1	59.7	32.9	3.8	1.2	1.5	0.4	0.2	0.4
R - 2	56.9	31.4	5.3	2.0	2.7	0.2	0.4	1.1
*R - 3	73.0	19.2	3.5	0.9	1.3	1.0	0.6	0.5
Mean	63.2	27.8	4.2	1.4	1.8	-	0.4	0.7

*Stored in cold room at 40° F - other samples in freezer at -10° F.

TABLE 16.

Volatile Acids in Unclean (U) and Rancid (R) Cheddar Cheese in mg/kg Cheese

Sample	Volatile Acids							
	C2	C4	C6	C8	C10	C12	Unknown A	Unknown B
U - 1	1255	65	27	22	36	12	14	37
U - 2	1251	73	28	22	40	11	11	32
U - 3	789	76	33	22	21	-	11	82
U - 4	1108	433	97	49	89	37	11	33
U - 5	1074	71	32	25	37	13	8	39
*U - 6	1334	258	73	35	63	31	13	16
*U - 7	1824	134	50	30	57	18	6	18
Mean	1234	159	49	29	49	-	11	37
R - 1	749	606	93	35	53	17	6	14
R - 2	242	196	44	21	33	3	5	15
*R - 3	640	247	59	19	33	30	13	30
Mean	544	350	65	25	40	-	8	20

*Stored in cold room at 40° F - other samples in freezer at -10° F.

TABLE 17.

Volatile Acids in Unclean (U) Cheddar Cheese held in Cold Room and Freezer Storage in mg/kg Cheese

Sample		Volatile Acids							
		C2	C4	C6	C8	C10	C12	Unknown A	Unknown B
U-1	(Freezer	1255	65	27	22	36	12	14	37
	(Cold Room	1460	128	60	27	43	22	17	27
U-2	(Freezer	1251	73	28	22	40	11	11	32
	(Cold Room	1538	163	74	47	55	25	19	39
U-3	(Freezer	789	76	33	22	21	-	11	82
	(Cold Room	1544	191	66	45	68	27	6	31
U-4	(Freezer	1108	433	97	49	89	37	11	33
	(Cold Room	1308	810	139	62	94	36	6	23
Mean	(Freezer)	1101	162	46	29	47	15	12	46
Mean	(Cold Room)	1463	323	85	45	65	28	12	30

TABLE 18.

Fat Test, Fat Acidity and Volatile Acids in University of Alberta (UA) Pasteurized-Milk Cheddar Cheese

Sample	Days from Date of Manufacture	Fat Test %	Fat Acidity mmoles per 100 g fat	Volatile Acids mmoles per 100 g cheese
UA - 1	34	33.0	0.90	1.29
	91		-	1.86
	170		1.46	2.51
*UA - 2	34	33.5	0.86	0.93
	91		-	1.44
	170		1.26	1.58
UA - 3	36	32.0	0.84	0.95
	93		-	1.21
	170		1.60	1.69
*UA - 4	36	32.0	1.01	1.12
	93		-	1.74
	170		1.77	2.47
UA - 5	18	35.5	0.96	0.76
	93		-	0.77
	126		-	1.12
	153		0.94	1.19
Mean of 1 month old cheese.....			0.91	1.00
Mean of 3 months old cheese.....			-	1.40
Mean of 5-6 months old cheese.....			1.41	1.89

* 10% Raw milk was added.

TABLE 19.

Volatile Acids in University of Alberta (UA)
Pasteurized-Milk Cheddar Cheese
in Mole %

Sample	Days From Date of Manufacture	Volatile Acids								
		C2	C3	C4	C6	C8	C10	C12	Unknown A	Unknown B
UA - 1	34	93.1	-	3.5	0.8	0.7	1.2	-	0.2	0.5
	91	92.9	-	2.9	0.7	0.4	0.8	0.7	1.2	0.4
*UA - 2	34	90.4	-	3.6	1.1	0.9	1.8	1.0	0.4	0.8
	91	91.4	-	3.3	1.1	0.8	1.0	0.6	1.0	0.8
	170	84.9	0.4	4.7	1.3	0.9	1.1	0.3	6.1	1.1
UA - 3	36	92.2	-	3.1	1.1	1.2	2.4	-	-	-
	93	90.5	-	4.0	1.4	1.1	1.5	0.3	0.8	0.3
*UA - 4	36	91.3	-	3.5	1.2	1.3	1.7	-	0.2	0.8
	93	92.4	0.1	3.7	1.1	0.7	1.0	0.4	0.4	0.4
	170	86.6	-	6.6	1.8	1.7	2.0	0.4	0.7	0.2
UA - 5	18	97.4	-	2.2	0.4	-	-	-	-	-
	93	91.2	-	2.1	1.0	1.4	2.7	0.6	0.3	0.6
	126	88.4	-	4.2	1.2	1.4	3.0	0.7	0.8	0.4
	153	73.1	16.0	4.1	2.1	0.6	0.6	-	3.7	-
Mean of 1 month old cheese.....		92.9	-	3.2	0.9	1.0	-	-	-	-
Mean of 3 months' old cheese.....		91.7	-	3.2	1.1	0.9	-	-	-	-
Mean of 5-6 months' old cheese.....		81.5	-	5.1	1.7	1.1	-	-	-	-

* 10% Raw milk was added.

TABLE 20.

Volatile Acids in University of Alberta (UA) Pasteurized-Milk Cheddar Cheese in mg/kg Cheese

Sample	Days From Date of Manufacture	Volatile Acids								
		C2	C3	C4	C6	C8	C10	C12	Unknown A	Unknown B
UA - 1	34	721	-	40	12	12	26	-	-	-
	91	1037	-	47	16	11	26	16	36	12
*UA - 2	34	504	-	30	12	13	29	19	5	14
	91	790	-	42	18	17	25	16	22	21
	170	805	5	66	24	21	29	8	153	9
UA - 3	36	526	-	26	12	16	39	-	-	-
	93	657	-	43	20	19	32	8	15	8
*UA - 4	36	613	-	35	16	21	32	-	4	17
	93	964	1	56	21	17	29	14	10	13
	170	1284	-	143	52	61	87	20	26	10
UA - 5	18	444	-	15	3	-	-	-	-	-
	93	421	-	14	9	16	36	10	4	9
	126	594	-	42	16	22	57	15	14	7
	153	522	141	43	28	10	11	-	69	-
Mean of 1 month old cheese.....		562	-	29	11	-	-	-	-	-
Mean of 3 months' old cheese.....		774	-	40	17	-	-	-	-	-
Mean of 5-6 months' old cheese.....		870	-	84	35	-	-	-	-	-

* 10% Raw milk was added.

TABLE 21.

Fat Test, Fat Acidity and Volatile Acids in Gouda, Edam, Swiss and Alberta Pasteurized-Milk Cheddar Cheese

Sample	Fat Test %	Fat Acidity mmoles per 100 g fat	Volatile Acids mmoles per 100 g cheese
Gouda	30.0	0.86	1.48
Edam	27.0	2.34	2.42
Swiss	16.0	2.98	8.52
Pasteurized - 1	34.0	0.84	0.40
Pasteurized - 2	28.0	1.34	2.12

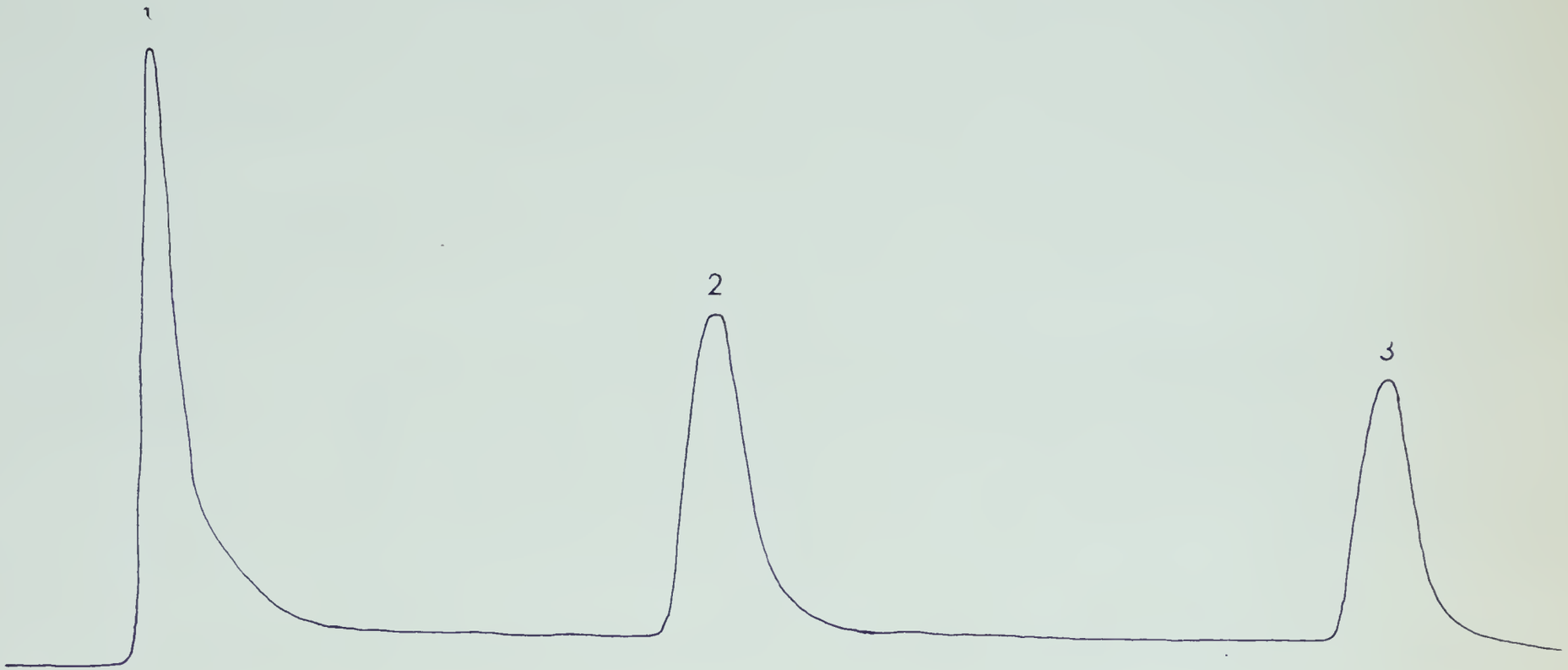

Fig. 4 Gas-chromatogram of volatile acids from sample 0 - 10 (aqueous portion): peak 1 - acetic acid, peak 2 - butyric acid, peak 3 - caproic acid.

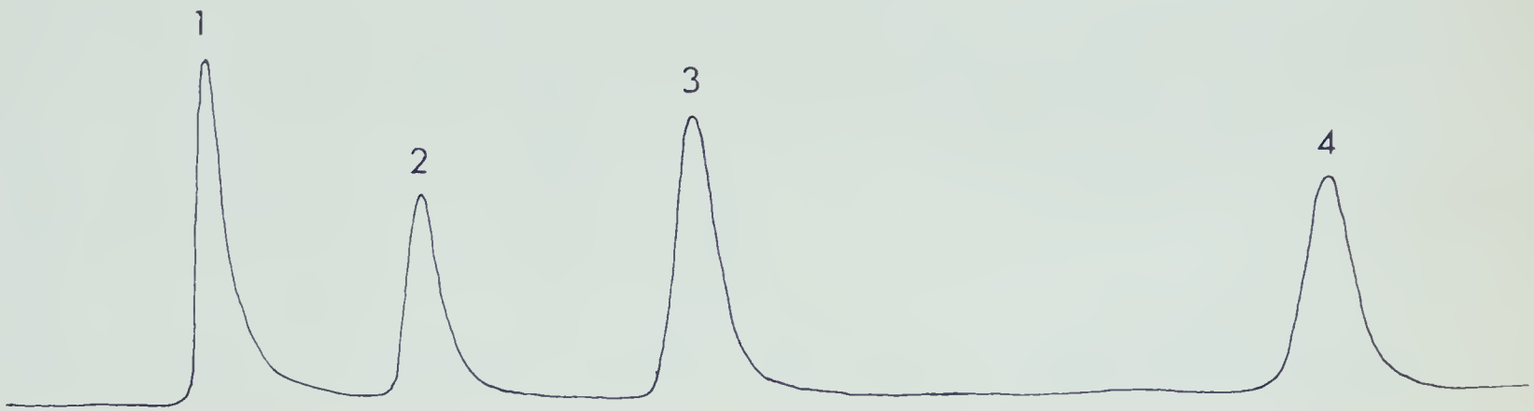

Fig. 5 Gas-chromatogram of volatile acids from sample 0 - 5 (aqueous portion): peak 1 - acetic acid, peak 2 - propionic acid, peak 3 - butyric acid, peak 4 - caproic acid.

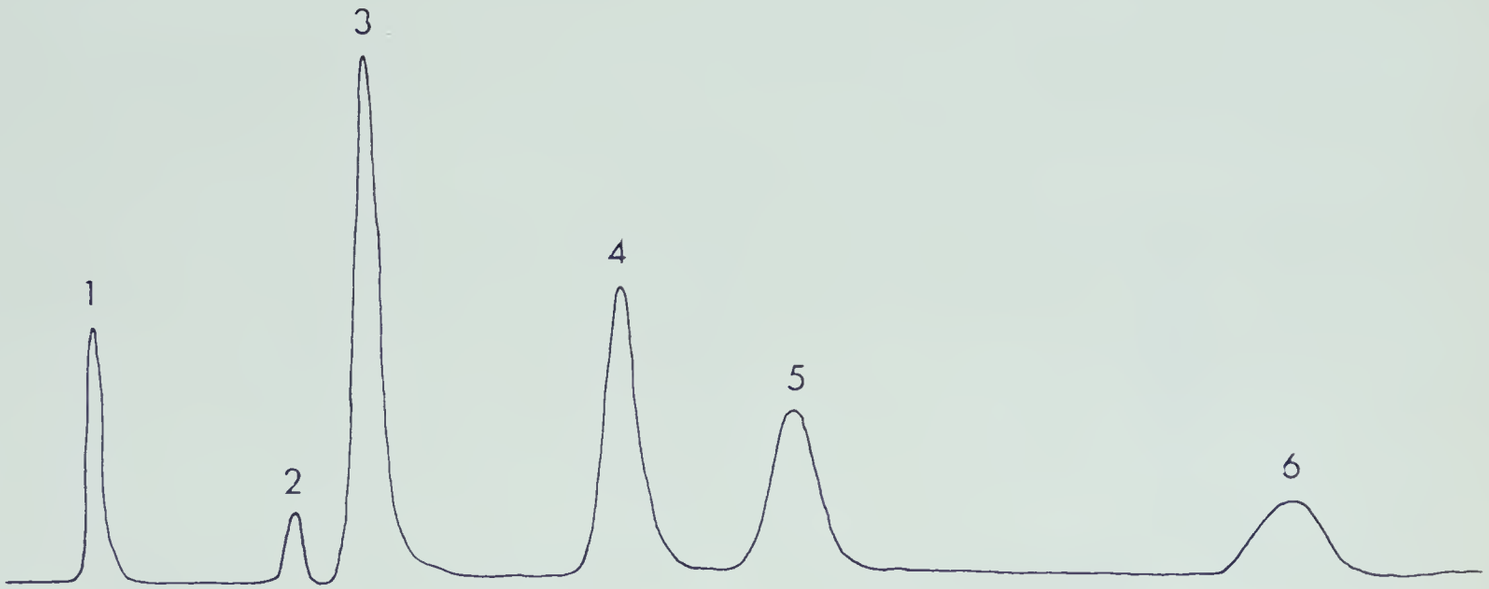

Fig. 6 Gas-chromatogram of volatile acids from sample 0 - 12 (CS_2 portion): peak 1 - caproic acid, peak 2 - caprylic acid, peak 3 - unknown A, peak 4 - capric acid, peak 5 - unknown B, peak 6 - lauric acid.

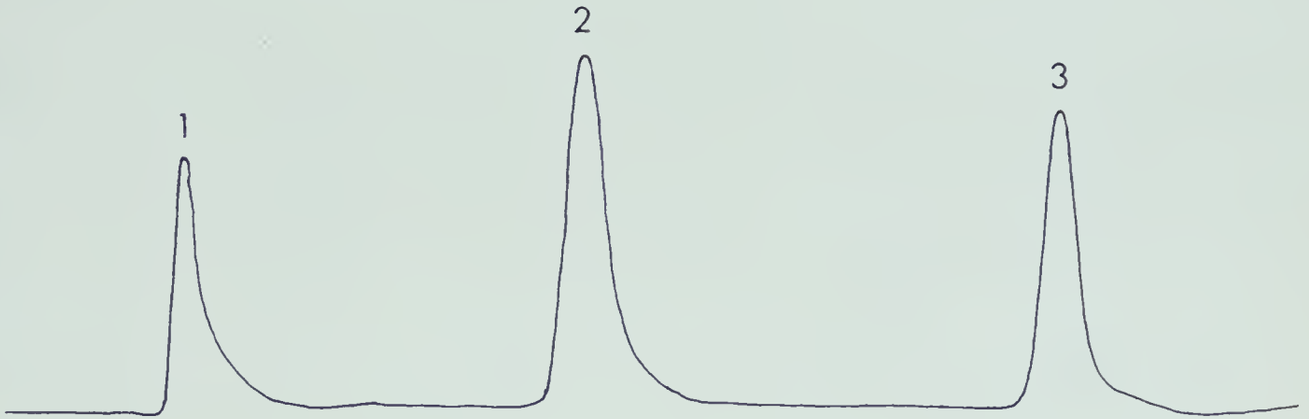

Fig. 7 Gas-chromatogram of volatile acids from sample R - 1 (aqueous portion): peak 1 - acetic acid, peak 2 - butyric acid, peak 3 - caproic acid.

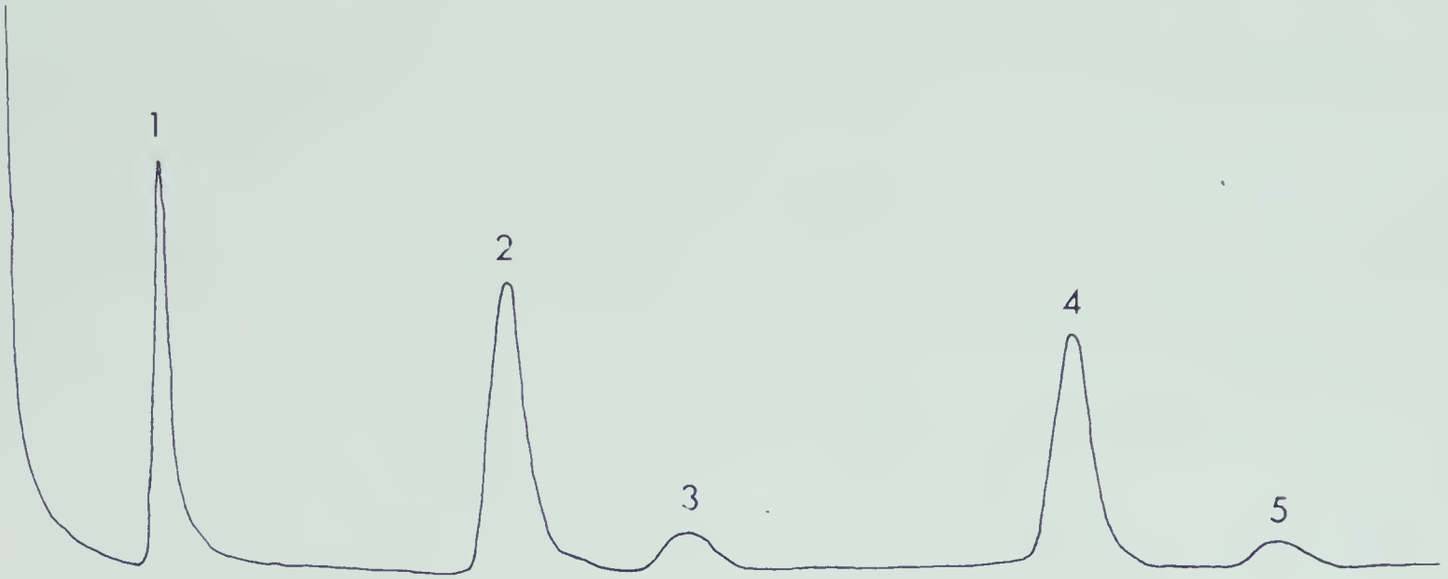

Fig. 8 Gas-chromatogram of volatile acids from sample R - 1 (CS_2 portion): peak 1 - caproic acid, peak 2 - caprylic acid, peak 3 - unknown A, peak 4 - capric acid, peak 5 - unknown B.

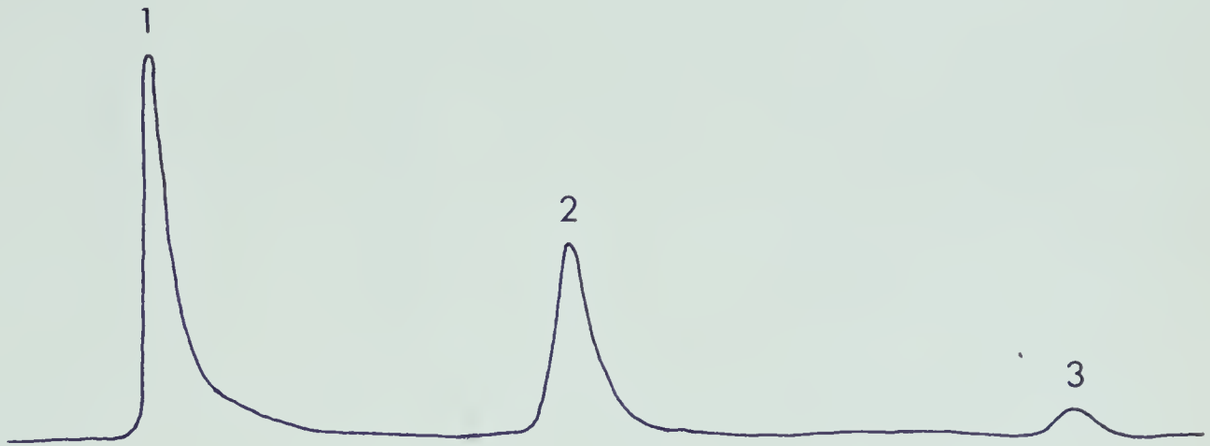

Fig. 9 Gas-chromatogram of volatile acids from 90 day old UA - 1 (aqueous portion): peak 1 - acetic acid, peak 2 - butyric acid, peak 3 - caproic acid.

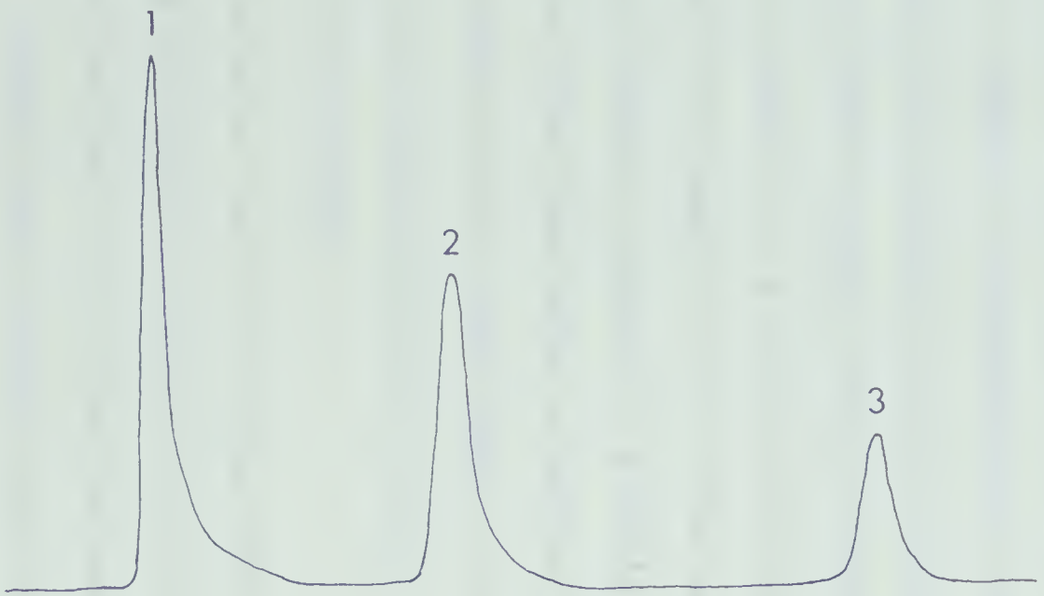

Fig. 10 Gas-chromatogram of volatile acids from 90 day old UA - 2 (aqueous portion): peak 1 - acetic acid, peak 2 - butyric acid, peak 3 - caproic acid.

DISCUSSION

I. Fat Content of Cheese.

The butterfat test of the commercial Cheddar cheese analyzed fell within a narrow range with a mean fat test of 34.8% (Table 4). This was to be expected as a minimum butterfat content is required by regulations under the Canada Dairy Products Act for Canadian Cheddar cheese. The minimum butterfat content required is now 50% on a moisture-free basis having been raised from 48% on August 17, 1966. The reason the fat content in this study has been reported on a cheese basis rather than on a moisture-free cheese basis is that the volatile acid analyses were carried out on a cheese basis. This was necessary as the lower volatile acids, being water soluble, are present in the aqueous portion of the cheese.

The butterfat test of the Gouda, Edam and Swiss cheeses showed a wide range of values. The butterfat test of the Swiss cheese was less than half the mean fat test of the Canadian Cheddar cheese. Swiss cheese is normally made from milk standardized to 3.0% butterfat, which would account for the lower test. The practice of skimming milk for cheese-making is common in the making of fancy (European) cheese.

II. Fat Acidity.

As cheese matures and ripening progresses, an increase in fat acidity would be expected due to lipolysis. While the

designations, mild, medium and old, used in the retail trade, are most likely based on flavor characteristics rather than on the actual age of the cheese, it would be reasonable to assume that the cheeses designated old were older than either the medium or mild cheeses. On the basis of the commercial Canadian Cheddar cheese analyzed, it would appear that the fat acidity of old cheese is approximately one-third higher than in the mild or medium cheeses (Table 4). The wide range of values in fat acidity in all three groups of cheeses points out the differing amounts of hydrolysis that occurred in cheeses of the same designation. It is suspected that some of the cheeses increased considerably in fat acidity from the time they were packaged for the retail trade until they were analyzed. It is also quite possible that storage and display temperatures accelerated fat acidity development.

The unclean samples stored in the cold room increased in fat acidity when compared to identical samples stored in the freezer where acid development was stopped (Table 14). These results show that hydrolysis of the fat in the cheese still occurs at cold room temperatures of 40° - 45° F, and that freezer storage of samples was necessary where any delay in analysis occurred. The fat acidity of the unclean and rancid samples (Table 14) was much lower than the mean fat acidity of the mild samples (Table 4). This is indicative of a lesser amount of fat hydrolysis taking place in the unclean and rancid cheeses stored in the freezer within one month of date of manufacture as compared to the mild samples which

would most probably be in excess of three months of age.

In the University cheese, the fat acidity increased from a mean of 0.91 mmoles per 100 g fat at one month of age to a mean of 1.41 at five to six months of age (Table 18). The much lower fat acidity in the University of Alberta pasteurized-milk cheese (Table 18) and the commercial pasteurized-milk cheese (Table 21) as compared to the fat acidity of commercial raw-milk Cheddar cheese (Table 4) emphasizes the pronounced effect of the pasteurization of the milk on fat hydrolysis. The addition of 10% raw milk of market milk quality as regards the microbial content apparently did not result in a sufficient change in the bacterial flora to materially alter the hydrolysis of the milk fat. It is a common observation that cheese made from a milk of low microbial content has a ripening pattern similar to that of a cheese made from pasteurized milk.

III. Volatile Acids.

The volatile acids resulting from lipolysis of the fat in cheese were the principal object of study in this investigation. The fact that acids up to and including caproic are water soluble and extremely volatile has made accurate analysis by investigators very difficult and delayed the accumulation of data on the hydrolysis of milk fat during cheese ripening. Studies on the volatile acids of cheese that have been reported in the literature indicate the use of methods employing principles which are open to criticism.

Methods involving solvent extraction of the fat and free fatty acids only partially recover the water soluble fatty acids. Column chromatographic techniques do not completely prevent volatile acid losses, particularly butyric, during analysis. The two-step gas-chromatographic procedure used in this investigation ensures the accurate determination of these acids for the reasons outlined in the experimental procedure. Comparison of the results of this study with those of two recent reports indicates that recovery of the lower molecular weight fatty acids is higher. Bills and Day (4a) report a mean level of acetic acid of 856 mg/kg of cheese in six samples of raw-milk Cheddar. The range of acetic acid was from 275 to 1316 mg/kg of cheese. Butyric acid showed a range of from 80 to 207 with a mean of 128, and caproic acid a range of from 35 to 79 with a mean of 48. Kristoffersen, et al. (28a) report a study of 24 lots of Cheddar cheese, 4 of which were raw-milk Cheddar. These four cheeses showed a mean level of acetic acid of 1317 mg/kg of cheese with a range of from 600 to 2560. The butyric acid content showed a range of from 140 to 180 with a mean of 165 mg/kg of cheese. The mean butyric acid content in the 43 samples analyzed by the two-step gas-chromatographic procedure in the present investigation was 232 mg/kg of cheese which was 104 mg higher than the figure reported by Bills and Day and 67 mg higher than that reported by Kristoffersen, et al. While a direct comparison is not valid because of different cheeses

being analyzed in all three cases, the results of this study suggest that the method used gives a more accurate analysis of the lower molecular weight fatty acids.

The presence of two unidentified volatile acids was observed in most of the commercial cheeses. Unknown A appeared between caprylic and capric and Unknown B between capric and lauric. No previous reference to the presence of these acids has been noted in the literature. In unpublished data from the Department of Dairy and Food Science, University of Alberta, Edmonton, similar acids were found in a number of cheeses while conducting a study on partial glycerides. It has been postulated that these unknown acids could be decomposition products resulting from fission at the double bond of higher molecular weight fatty acids. The presence of these acids was also noted in the one-month old University of Alberta cheese, indicating that their development can occur in quite immature cheese.

The marked increase in butyric and caproic acid levels from mild to old cheese and the somewhat lesser increase in caprylic and capric, is indicative of the increased fat hydrolysis occurring with the increased age of the cheese. Flavor intensity in the samples analyzed was noted as being considerably stronger in the old samples when compared to the mild. This suggests that increased levels of butyric and caproic, and possibly caprylic and capric acids contribute to the flavor. The data also indicates that the ratio of butyric acid to acetic acid, in the old cheeses analyzed

was approximately 1:3, whereas, in the mild cheese, the ratio was about 1:6. Because of the low flavor threshold of the lower molecular weight fatty acids and, in particular, butyric, there would appear to be justification for suggesting that the flavor characteristics in the commercial cheese analyzed were influenced by:

(1) the amount of lower molecular weight free fatty acids (C4 to C8) and particularly butyric acid, and

(2) the ratio of lower molecular weight free fatty acids (C4 to C8), particularly butyric, to the acetic acid level.

The distribution of volatile acids and their level in the unclean samples (Table 17) was similar to the mean of the mild samples (Table 12). Hlynka, et al. (18) reported the development of an unclean flavor in cheese made from milk which had been severely agitated, and they postulated this condition reveals the early development of rancidity. On the basis of the cheese analyzed in this study, the official government grading of unclean would not appear to be invariably associated with the butyric acid level. It is noteworthy however, that the unclean samples held in the cold room for approximately seven months developed a butyric acid level equal to the mean of the old cheeses.

In the rancid samples analyzed there was as much butyric acid present as in the old samples, but only half as much acetic

acid. The ratio of butyric acid to acetic acid was approximately 1.0:1.5. The organoleptic grading of rancid by the official government graders is an indication that the high level of butyric acid in association with the relatively low level of acetic acid was sufficiently pronounced to be objectionable. It was unfortunate that only three rancid samples were available for analysis; but they gave a very strong indication of a high ratio of butyric and caproic acids as compared to the acetic acid level. Further analyses of rancid cheeses to determine levels and ratios of low molecular weight volatile acids would be advantageous.

The low volatile acidity and the relatively high levels of acetic acid in the cheese made at the University of Alberta from pasteurized milk, confirms that there is a reduction of fat hydrolysis in cheese made from pasteurized milk, as has been reported by many investigators.

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